

The Effect of Raw Soybean on the Pancreas of Adult Dogs¹ (35511)

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(Introduced by E. L. Smith)

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Soybeans offer an abundant source of high quality protein, but active antinutritional factors in uncooked soybeans limit their use for human consumption. Young rats (1) and chicks (2) fed raw soybean develop pancreatic hypertrophy, hypersecretion of pancreatic enzymes, and growth depression; however, adult rats (3) and chickens (4) are less severely affected by the diet. Gorrill and Thomas (5) and Hooks *et al.* (6) found that calves and growing pigs fed raw soybean did not develop pancreatic hypertrophy or hypersecretion of pancreatic enzymes. These results suggested that some of the pancreatic effects of raw soybean might be dependent on the age and species of animal used.

The purpose of the present study was to investigate the effect of raw soybean on some physiological and biochemical parameters of the pancreas of adult dogs.

Materials and Methods. Adult male and female mongrel dogs ranging in body weight from 6.1 to 19.1 kg were selected at random for these experiments. Eight dogs were used as controls and were fed daily 16.9 g Canine Maintenance Science Diet (Topeka, Kans.)/kg of body weight while 30 dogs received daily a 15% raw soybean diet which contained 14.4 g of Canine Maintenance Science Diet and 2.5 g of raw soybean flakes (Central Soya, Chicago, Ill.)/kg of body weight. An additional six dogs were fed daily

a 30% raw soybean diet consisting of 11.9 g of Canine Maintenance Science Diet and 5.0 g of raw soybean flakes/kg of body weight.⁴ The weight of each dog was determined twice each week.

The control dogs were fed the diet for 2 weeks, fasted 24 hr after the final feeding period, anesthetized with sodium pentobarbital and the pancreas of each dog was then removed and placed on ice prior to killing the dog. The dogs fed 15% raw soybean were sacrificed in groups of 6 after 2, 3, 5, 8, and 12 weeks, and the 6 dogs fed 30% raw soybean were killed after 8 weeks on the diet by the same procedure used for killing the control dogs. Histological sections, from the head, neck, and tail of each pancreas were made. The pancreases were weighed and homogenized at 4° in approximately 10% (w/v) of 0.15 M NaCl containing 0.1% Triton X-100.

Triplicate samples of each pancreatic homogenate were diluted to a concentration of protein between 0.35 to 0.70 mg of protein/ml as determined by the method of Lowry *et al.* (7) and the samples were assayed for trypsinogen and chymotrypsinogen by the method of Gorrill and Thomas (8) using tosyl arginine methyl ester (TAME) and benzoyl tyrosine ethyl ester (BTEE) dissolved in 26% methanol as the respective substrates. The diluted homogenates were incubated with 0.5% purified enterokinase (CalBiochem, Los Angeles, Calif.) for 1 hr at 37° in the presence of CaCl₂ to convert trypsinogen and chymotrypsinogen to their active forms prior to being assayed. The pan-

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⁴ The diets used were compounded to meet the daily requirements for maintenance of adult dogs following nutritional counseling with Dr. Carl Schultz, Associate Director of Institutional Products, manufacturers of Science Diets.

TABLE I. The Effect of Raw Soybean on Pancreatic Size and Protein Content.^a

Length of feeding (weeks)	Diet	No. of dogs	Body wt (kg)	Pancreas wt (g)	Pancreas (g/kg of dog)	Protein (mg/g of pancreas)
2	Control	8	I 13.64	26.63 ± 1.92 ^b	1.98 ± 0.09	175.96 ± 14.92
			T 13.64			
2	15% RS	6	I 12.26	25.70 ± 1.71	2.29 ± 0.14	166.41 ± 4.90
			T 11.72			
3	15% RS	6	I 13.12	26.30 ± 0.92	2.18 ± 0.07	161.68 ± 9.18
			T 12.77			
5	15% RS	6	I 12.68	22.52 ± 1.12	2.12 ± 0.22	184.14 ± 5.50
			T 11.81			
8	15% RS	6	I 10.09	20.53 ± 1.25	2.22 ± 0.14	167.69 ± 7.03
			T 9.62			
12	15% RS	6	I 8.49	19.98 ± 1.58	2.55 ± 0.18 ^c	170.10 ± 6.93
			T 8.13			
8	30% RS	6	I 10.52	23.25 ± 2.54	2.39 ± 0.28	174.03 ± 10.34
			T 10.06			

^a I = initial wt; T = terminal wt; and RS = raw soybean.

^b Mean ± SE.

^c Significance $p < .01$ compared to the control.

creatic homogenates were assayed for amylase by the method of Bernfield (9) and lipase was determined by a modification of the method of Marchis-Mouren (10) using trioctanoin as the substrate. RNA and DNA were determined by the methods of Hurlbert *et al.* (11) and Burton (12), respectively. The raw soybean used in compounding the 15 and 30% diets was assayed for trypsin inhibitor by the method of Kakade *et al.* (13).

Data were analyzed for statistical significance by the Mann-Whitney *U* test (14).

Results. Dogs fed the 15% soybean diet which contained 1273 units of trypsin inhibitor/kg as well as dogs fed 30% soybean which contained 2546 units of trypsin inhibitor had no significant change in body weight over the experimental period (Table I).

No significant histological difference was seen between the pancreases of control dogs and those fed 15 and 30% soybean with the possible exception of a widening of the ergastoplasmic zone of the acini of the dogs on the soybean diet. The latter observation suggested that there might be a higher rate of protein synthesis in the dogs on the soybean diet, but upon biochemical analyses of the

tissue protein there was no evidence of a significant increase in protein synthesis in these dogs compared to the control dogs (Table I). The RNA concentration (mg/g of pancreas) was significantly decreased ($p < .05$) in dogs fed 15% soybean and further decreased ($p < .01$) in dogs fed 30% soybean (Table II). Therefore, if RNA analyses are any indication of protein synthesis, it appears that the soybean diet caused a decrease rather than an increase in protein synthesis.

For dogs on the 15% soybean diet, there was no evidence of pancreatic enlargement (g of pancreas/kg of body wt until week 12 when they were significantly heavier ($p < .01$) than those of the controls (2.55 vs 1.98 g of pancreas/kg of body wt, Table I). The dogs fed on the 30% soybean diet for 8 weeks showed no evidence of pancreatic enlargement. The DNA concentration (mg/g of pancreas) of dogs fed either on the 15 or 30% soybean diet was not significantly different from that of the control dogs. Previous investigators (15) have assumed that the DNA level is constant per cell for normal cells; therefore, the increase (g of pancreas/kg) observed in week 12 would indi-

TABLE II. The Effect of Raw Soybean on Nucleic Acids of the Pancreas.

Length of feeding (weeks)	Diet	No. of dogs	RNA (mg/g of pancreas)	DNA (mg/g of pancreas)
2	Control	8	20.95 ± 0.44 ^a	2.38 ± 0.13
2	15% RS ^b	6	18.93 ± 1.20	2.27 ± 0.08
3	15% RS	6	18.37 ± 0.99 ^a	2.09 ± 0.04
5	15% RS	6	19.07 ± 0.59 ^a	2.03 ± 0.13
8	15% RS	6	19.29 ± 0.15 ^a	2.03 ± 0.10
12	15% RS	6	19.31 ± 0.55 ^a	2.15 ± 0.14
8	30% RS	6	17.26 ± 0.89 ^a	2.35 ± 0.22

^a Mean ± SE.

^b RS = raw soybean.

^c Significance compared to the control $p < .05$; ^d $p < .01$.

cate an increase in the number of cells since the DNA did not change. However, no evidence of hyperplasia was observed in the histological sections.

Trypsinogen activity was significantly decreased ($p < .01$) after 2 and 3 weeks on the 15% soybean diet with the lowest activity (675.35 units/g of pancreas) observed during week 5 (Table III). After week 5, the trypsinogen activity increased gradually until by week 12 there was no significant difference in activity between the treated and control dogs. The trypsinogen activity of dogs on the 30% soybean diet for 8 weeks was significantly lower ($p < .01$) than that of the control dogs, 981.42 vs 1644.17 units/g of pancreas (Table III).

The effect of the 15% soybean diet on chymotrypsinogen activity was similar to that of trypsinogen with a significant decrease ($p < .05$) in week 2 compared to the control dogs (Table III). The activity continued to decrease ($p < .01$) compared to the control through week 5; however, the chymotrypsinogen activity of subsequent groups gradually increased until, during week 12, there was no significant difference between the control and treated dogs. Contrary to the observation with trypsinogen there was no significant difference in the chymotrypsinogen activity between control dogs and those on the 30% soybean diet 8 weeks (Table III).

A significant decrease ($p < .01$) in the lipase activity from control values was observed for dogs fed for 2 weeks on 15% soybean (13.94×10^2 vs 43.00×10^2 units/g

of pancreas, Table III). The lowest level of lipase activity, 8.14×10^2 units/g of pancreas, occurred during week 8 after which it began to increase and by week 12 the lipase activity was not significantly different from that of the control dogs. The lipase activity of dogs on the 30% soybean diet for 8 weeks was not affected.

There was no measurable effect on the pancreatic amylase activity of dogs on either the 15 or 30% soybean diet.

Discussion. The extensive pancreatic hypertrophy which was observed previously in young rats (1) and chicks (2) fed comparable levels of soybean over shorter experimental periods was not observed in these experiments on adult dogs. Although dogs fed 15% soybean for 12 weeks did have significantly more grams of pancreas per kilogram than control dogs still the heaviest organ to weight ratio of our treated groups was well within the normal range for dogs reported by Jackson and Capiello (16); further, there was no evidence of pancreatic enlargement in our dogs fed twice as much soybean (30%). The dog's resistance to the pancreatic hypertrophy factor(s) present in raw soybean appears then to be similar to that reported for calves (5) and growing pigs (6).

The level of trypsinogen, chymotrypsinogen, and lipase were depressed in dogs fed 15% soybean 2, 3, 5, and 8 weeks, but in those dogs sacrificed after week 12, the enzymatic activity was the same as that of control dogs. Apparently adaption of the pancreas to the dietary stimulus took place between

TABLE III. The Effect of Raw Soybean on Pancreatic Enzymes.

Length of feeding (weeks)	Diet	No. of dogs	Trypsinogen (units/g of pancreas; units/mg of protein)	Chymotrypsinogen (units/g of pancreas; units/mg of protein)	Lipase (units/g of pancreas $\times 10^3$; units/mg of protein)	Amylase (units/g of pancreas $\times 10^3$; units/mg of protein)
2	Control	8	1644.17 \pm 145.06 ^a 9.42 \pm 0.50	365.35 \pm 35.79 2.07 \pm 0.10	43.00 \pm 3.92 26.09 \pm 3.34	8.02 \pm 0.96 49.20 \pm 8.34
2	15% RS ^b	6	837.05 \pm 70.95 ^c 5.00 \pm 0.33	231.66 \pm 38.65 ^d 1.37 \pm 0.21	13.94 \pm 3.21 ^c 8.22 \pm 1.84	7.87 \pm 1.43 47.03 \pm 8.04
3	15% RS	6	782.33 \pm 68.94 ^c 4.85 \pm 0.41	229.48 \pm 36.13 ^d 1.38 \pm 0.17	15.13 \pm 4.97 ^c 9.50 \pm 3.18	8.23 \pm 1.44 49.56 \pm 6.19
5	15% RS	6	675.35 \pm 61.55 ^c 3.66 \pm 0.29	148.32 \pm 31.72 ^c 0.80 \pm 0.16	17.48 \pm 6.09 ^c 9.40 \pm 3.15	7.80 \pm 1.94 41.41 \pm 9.90
8	15% RS	6	839.25 \pm 53.91 ^c 4.99 \pm 0.13	160.83 \pm 19.59 ^c 0.95 \pm 0.09	8.14 \pm 1.53 ^c 4.75 \pm 0.74	8.75 \pm 1.42 51.11 \pm 6.78
12	15% RS	6	1137.19 \pm 152.75 6.62 \pm 0.78	241.96 \pm 46.33 1.40 \pm 0.25	33.17 \pm 9.23 19.31 \pm 5.31	8.66 \pm 2.11 49.38 \pm 10.78
8	30% RS	6	981.42 \pm 119.36 ^c 5.64 \pm 0.65	250.17 \pm 29.03 1.43 \pm 0.13	51.13 \pm 13.30 27.94 \pm 5.60	10.06 \pm 1.20 57.45 \pm 5.60

^a Mean \pm SE.^b RS = raw soybean.^c Significance compared to the control: $p < .01$; ^d $p < .05$.

weeks 8 and 12. These results differ from the increased levels of these enzymes reported by other investigators in chicks and rats (17, 18). However, these latter studies were done with young animals over shorter experimental periods. The decrease in enzyme activity observed in our study probably was not due to an increased pancreatic secretion which would have depleted the acini since the animals were fasted 24 hr before being sacrificed. We have previously found in other studies with adult dogs that soybeans did not increase the secretion of pancreatic enzymes as reported in studies with rats and chickens (1, 2). Instead, a 15% soybean diet must have caused a temporary decrease in the synthesis of trypsinogen, chymotrypsinogen, and lipase which would account for the observed decrease in enzyme activity. The decreased synthesis of these enzymes may have resulted from a deficiency of essential amino acids since soybeans were reported not only to be deficient in methionine, valine, and threonine, but to contain factor(s) interfering with metabolism of these amino acids (19, 20). Gorrill and Thomas (5) previously suggested there may have been a decreased synthesis of enzymes causing a reduction in the enzyme activity of pancreatic juice from calves fed soy flour milk. However, amylase may not have as high a demand for the limiting essential amino acids as the other enzymes assayed since the activity of this enzyme was not affected by the diet in our study. The dogs sacrificed after weeks 5 or 8 had begun adapting to the diet by obtaining from other body reservoirs the deficient essential amino acids for normal enzyme synthesis.

Dogs fed 30% soybean for 8 weeks had significantly less trypsinogen activity than control dogs, but the other enzymes were not significantly different. These results suggest that either: (i) The pancreas adapted more rapidly to the 30% soybean diet than dogs fed 15% soybean so that normal enzymes synthesis, with the exception of trypsinogen, was already reestablished, or (ii) the increased concentration of soybean, in spite of the defi-

ciency in 3 essential amino acids, was sufficient when fed in larger amounts to maintain adequate synthesis of all the enzymes except trypsinogen.

Summary. In summary, 15 or 30% dietary soybean did not significantly alter the cellular structure or weight of the pancreas (g/kg) of the adult dog. The synthesis of trypsinogen, chymotrypsinogen, and lipase was temporarily impaired, but their synthesis was returned to normal. The effects of raw soybean on the dogs in this study were different from those reported in rats and chicks.

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